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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A01N 63/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 96/39840</b> <b>(43) International Publication Date:</b> 19 December 1996 (19.12.96)
<b>(21) International Application Number:</b> PCT/US96/08553 <b>(22) International Filing Date:</b> 4 June 1996 (04.06.96) <b>(30) Priority Data:</b> 08/484,784      7 June 1995 (07.06.95)      US <b>(71) Applicant:</b> THE O'DONNELL FAMILY TRUST [US/US]; 1145 Linda Vista Drive, San Marcos, CA 92009 (US). <b>(72) Inventor:</b> O'DONNELL, Boyd; 1145 Linda Vista Drive, San Marcos, CA 92009 (US). <b>(74) Agents:</b> SCHNEIDER, Carol, A. et al.; Lyon & Lyon, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).		<b>(81) Designated States:</b> CA, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished          upon receipt of that report.</i>
<b>(54) Title:</b> TREATMENT OF SOIL AND PLANTS WITH A COMPOSITION CONTAINING BACILLUS LATEROSPORUS  <b>(57) Abstract</b> <p>A method for treating soil with a composition containing the microorganism <i>Bacillus laterosporus</i> strain BOD is disclosed. Treatment of the soil with <i>B. laterosporus</i> strain BOD results in certain beneficial changes to the soil including maintenance of an alkaline pH, fixation of plant nutrients, neutralization of odors, a reduction in aerobic and coliform bacterial counts, and inhibition of plant pathogenic bacteria and fungi. A method for treating plants with <i>B. laterosporus</i> strain BOD to inhibit the growth of plant pathogenic organisms is also disclosed.</p>		

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DESCRIPTIONTreatment of Soil and Plants With a  
Composition Containing *Bacillus laterosporus*Related Applications

This application is a continuation-in-part of application Serial No. 08/236,701 filed April 28, 1994, which is a continuation of application Serial No. 07/908,631  
5 filed July 1, 1992, now abandoned, which is a continuation of application Serial No. 07/621,603 filed Dec. 4, 1990, now abandoned.

Technical Field

The present invention relates generally to a method  
10 for treating soil to improve the soil for agronomic purposes and for other purposes. The present invention also relates to a method of treating plants to control certain plant pathogenic organisms.

Background Art

15 *Bacillus laterosporus* was not previously known for use in treating soil. A previously known method for increasing the alkalinity of the soil is to add lime. The ability to maintain an alkaline soil pH with *Bacillus laterosporus* is the equivalent to the use of tons of lime  
20 per acre per year. *Bacillus laterosporus* was not previously known for use in controlling plant pathogenic organisms.

Summary of the Invention

The present invention is directed to methods for  
25 treating soil and plants, using a composition comprising the microorganism *Bacillus laterosporus* strain BOD.

In one separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus* strain BOD to maintain an alkaline pH is contemplated.

In a further separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus* strain BOD to reduce the aerobic bacterial count is contemplated.

5 In a further separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus* strain BOD to reduce soil odor is contemplated.

In a further separate aspect of the present invention, a method of treating soil with *Bacillus laterosporus*  
10 strain BOD to inhibit plant pathogenic organisms is contemplated.

In a further separate aspect of the present invention, a method of treating plants with *Bacillus laterosporus* strain BOD to inhibit plant pathogenic organisms is  
15 contemplated.

Accordingly, an object of the present invention is to provide methods for treating soil and plants with *Bacillus laterosporus* strain BOD.

#### Detailed Description of the Invention

20 Treatment of soil with a composition containing *B. laterosporus* strain BOD results in a number of beneficial changes for agronomic purposes. The *B. laterosporus* strain BOD that are added to the soil maintain the pH level of the soil in the alkaline range. The ability to  
25 perform this function is equal to tons of lime per acre per year. The maintenance of the soil in the alkaline range has a number of advantages. These include fixing soil nutrients by preventing the acidification of the soil and the resulting leaching of nutrients. Ammonia nitrogen  
30 which is easily lost into the air is converted in alkaline pH soil to nitrate nitrogen which is fixed in the soil and easily assimilated by plants. Potassium is more insoluble at high pH and therefore less likely to be leached out by rain. The *B. laterosporus* strain BOD was also effective  
35 in eliminating odors. This anti-odor effect was probably

due, at least partially, to the maintenance of a more alkaline pH.

The *B. laterosporus* strain BOD composition is also effective in altering the microbial composition of the soil. These alterations include decreasing the overall number of aerobic bacteria. The reduction in bacteria include a reduction in *Salmonella* species, *Klebsiella* species, *Escherichia coli*, *Staphylococcus* species and total coliform species. The reduction of these bacterial populations indicates a reduction in potentially pathogenic microorganisms. The probable mechanism by which these changes occur is through the maintenance of an alkaline pH, which inhibits the growth of acid bacteria. Other possible factors are the production of metabolites by the *B. laterosporus* which interfere with coliform growth or the competition between *B. laterosporus* strain BOD and the pathogenic bacteria.

*B. laterosporus* strain BOD was originally isolated from a soil sample from Iceland and was selected for further research based on its ability to inhibit pathogenic bacteria in soil and lab test media. The examples described herein utilize *B. laterosporus* strain BOD to treat soil. Table I below shows the results obtained when soil was treated with *B. laterosporus* strain BOD. Treated soil and untreated soil were compared 30 days after treatment.

TABLE I

	ANALYSIS	CONTROL	30 DAYS	% CHANGE
	aerobic bacteria plate count	52x10 <sup>6</sup> /gm	30x10 <sup>6</sup> /gm	- 42%
5	total coliform bacteria	460/gm	23/gm	- 95%
	E. coli (fecal coliform)	460/gm	<3/gm	- 99%
10	coagulate positive Staph.	23/gm	<3/gm	- 87%
	Salmonella detection	pos./25 gm	neg./25 gm	- 99%
	Klebsiella detection	pos./25 gm	neg./25 gm	- 99%
15	hydrogen ion (pH)	7.75 pH	8.45 pH	+ 9%
	total kjeldahl nitrogen	1,095 ppm	1,235 ppm	+ 13%
	potassium	7,372 ppm	8,319 ppm	+ 13%
20	phosphorous	510 ppm	524 ppm	+ 3%

In another experiment in the laboratory, *B. laterosporus* strain BOD was shown to be effective in inhibiting the growth of certain plant pathogenic bacteria and fungi in culture. The bacteria include *Clavibacter michiganense*,  
 25 *Erwinia carotovora*, *Erwinia chrysanthemi*, *Pseudomonas solanacearum*, *Pseudomonas syringae*, and *Xanthomonas campestris*. The fungi include *Aspergillus* species, *Bipolaris* species, *Cephalosporium* species, *Chaetomium* species, *Colletotrichum magna*, *Fusarium oxysporum*, *Penicillium* species,  
 30 *Phytophthora cinnamomi*, *Phytophthora citricola*, *Phytophthora citrophthora*, *Phytophthora parasitica*, *Pythium aphanidermatum*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Verticillium albo-atrum*, *Verticillium dahliae*, and *Verticillium* species.  
 35 Because *B. laterosporus* strain BOD was shown to be effective in the laboratory for inhibiting the growth of the above described species, it is expected that the

application of *B. laterosporus* strain BOD to the soil will be effective as a soil treatment to inhibit certain plant pathogenic organisms, including bacteria and fungi, and thereby reduce or eliminate certain plant diseases.

5 Additionally, it is expected that the application of *B. laterosporus* strain BOD directly to plants will be an effective means for controlling certain plant pathogenic bacteria and fungi.

#### Application

10 *B. laterosporus* strain BOD can be mixed with water and applied to the soil in the normal course of irrigation, including spray irrigation, drip irrigation, or any other means of irrigation. Alternatively, it can be applied independently of irrigation, i.e soil injection, topical

15 application. Effective ranges for application are between about  $3.785 \times 10^9$  cells per acre and  $3.785 \times 10^{12}$  cells per acre. The most effective range would be expected to be between about  $3 \times 10^{10}$  cells per acre and  $2 \times 10^{11}$  cells per acre. Generally, a relatively high initial application

20 will be followed by periodic applications thereafter, for as long as necessary to maintain the desired effects. The bacteria could also be applied to the soil as a mixture with other soil additives such as fertilizers, herbicides, or pesticides. The bacteria could also be applied in a

25 dry form such as in a powder, either alone or in formulation with other inert or active ingredients.

#### Best Mode For Carrying Out the Invention

The preferred method and composition for treating soil with *B. laterosporus* strain BOD consists of using *B.*

30 *laterosporus* strain BOD at a concentration of 10 million cells per milliliter of water. The water/bacteria mixture is applied to the soil by irrigation spraying at an initial rate of two gallons per acre and then applied periodically at a rate of one gallon per acre. Therefore,

35 the initial application is with  $7.57 \times 10^{10}$  cells per acre,

and the periodic application thereafter is with  $3.785 \times 10^{10}$  cells per acre. The soil is treated periodically for as long as necessary to maintain the desired effects.

*Bacillus laterosporus* strain BOD has been deposited  
5 for 30 years at the American Type Culture Collection  
(ATCC) in Rockville, Maryland and assigned Accession  
Number ATCC 55122. The present invention is not to be  
limited in scope by the organism deposited, since the  
deposited organism is intended to serve only as an example  
10 of one strain of *B. laterosporus* that would be effective  
in carrying out the invention. The term "*Bacillus latero-*  
*sporus* strain BOD", for the purposes of this invention, is  
intended to mean any strain of *Bacillus laterosporus* that  
is effective in treating soil and plants as described  
15 herein.

Variations on the specific illustrations of the  
invention disclosed herein will be apparent to those  
skilled in the art, and it is intended that this invention  
be limited only by the scope of the appended claims.



Claims

1. A method for improving the quality of soil comprising treatment of the soil with a composition comprising *Bacillus laterosporus* strain BOD.
- 5        2. A method according to claim 1 wherein said treatment further comprises application of *Bacillus laterosporus* strain BOD to the soil in an amount of between about  $3 \times 10^{10}$  cells per acre and  $2 \times 10^{11}$  cells per acre.
- 10        3. A method according to claim 1 wherein said treatment results in an increase in soil pH.
4. A method according to claim 1 wherein said treatment results in a soil pH of between about 7.5 and 8.5.
5. A method according to claim 1 wherein said treatment results in a soil pH of between about 8.0 and 8.25.
- 15        6. A method according to claim 1 wherein said treatment results in a reduction of soil odor.
7. A method according to claim 1 wherein said treatment results in a reduced count of aerobic bacteria.
8. A method according to claim 1 wherein said treatment results in a reduced count of coliform bacteria.
- 20        9. A method according to claim 1 wherein said treatment results in greater control of plant pathogenic bacteria.
10. A method according to claim 1 wherein said treatment results in greater control of plant pathogenic fungi.
- 25        11. A method of treating plants to inhibit the growth of plant pathogenic organisms comprising treatment of the

plants with a composition comprising *Bacillus laterosporus* strain BOD.

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<b>(54) Title:</b> TREATMENT OF SOIL AND PLANTS WITH A COMPOSITION CONTAINING BACILLUS LATEROSPORUS		
<b>(57) Abstract</b>  A method for treating soil with a composition containing the microorganism <i>Bacillus laterosporus</i> strain BOD is disclosed. Treatment of the soil with <i>B. laterosporus</i> strain BOD results in certain beneficial changes to the soil including maintenance of an alkaline pH, fixation of plant nutrients, neutralization of odors, a reduction in aerobic and coliform bacterial counts, and inhibition of plant pathogenic bacteria and fungi. A method for treating plants with <i>B. laterosporus</i> strain BOD to inhibit the growth of plant pathogenic organisms is also disclosed.		

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## INTERNATIONAL SEARCH REPORT

International Application No.

PL./US 96/08553

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A01N63/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 055 293 (ARONSON ET AL.) 8 October 1991 see claims ---	1-11
X	JOURNAL OF PHYTOPATHOLOGY, vol. 138, no. 3, 1993, pages 189-208, XP000610785 A.M. ROSALES ET AL.: "Identification of some bacteria from paddy antagonistic to several rice fungal pathogens " see page 189, the abstract see page 190, last paragraph - page 191, paragraph 1 see page 204, paragraph 3 see page 206, last paragraph - page 207 --- -/--	11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PESTICIDE SCIENCE, vol. 37, no. 4, 1993, BARKING, ESSEX, GB, pages 355-363, XP002019401 J.O. BECKER & F.J.SCHWINN: "Control of Soil-borne Pathogens with Living Bacteria and Fungi: Status and Outlook " see the whole document ---	1-11
A	US,A,4 663 162 (KADO ET AL.) 5 May 1987 see column 3, line 54 - column 5, line 5; claims 1-3 ---	1-11
A	US,A,4 952 229 (H.M. MUIR) 28 August 1990 see claims ---	1-10
A	CHEMICAL PATENTS INDEX, BASIC ABSTRACTS JOURNAL Week 8730 23 September 1987 Derwent Publications Ltd., London, GB; AN 87-210719 XP002019403 & JP,A,62 138 380 (BIOSTAR K.K.) see abstract ---	1-10
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A	TRENDS IN BIOTECHNOLOGY , vol. 7, no. 2, February 1989, CAMBRIDGE, GB, pages 39-44, XP002019402 J.W. KLOEPPER AT AL.: "Free-living bacterial inocula for enhancing crop productivity" see the whole document ---	1-10
A	US,A,4 582 704 (BAKER AT AL.) 15 April 1986 see claim 1 -----	11

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Information on patent family members

International Application No

PL./US 96/08553

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